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The Acquisition and Internalization of *Salmonella* by the Lesser Mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae)

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Abstract

In poultry broiler production facilities, it is important to understand the sources and contribution of reservoir populations of pathogens. The lesser mealworm beetle, *Alphitobius diaperinus* (Panzer), is a common pest in poultry litter that is reported to carry pathogens affecting both human and animal health. This study investigates whether the carriage of a bacterial pathogen occurs by the harboring of bacteria internally by these insects. Beetles were exposed to a marker bacterium, *Salmonella enterica* serovar Typhimurium-green fluorescent protein (ST-GFP), at concentrations up to 10^7 colony-forming units (cfu)/mL for 0.5 to 12 h, and then subsequently surface disinfected and dissected. The head, gastrointestinal tract and hemolymph were cultured for the presence of ST-GFP. This study definitively demonstrates the internal carriage of *Salmonella* by this insect and found that the beetles rapidly acquired bacteria from external sources and harbored the bacteria within their alimentary canal after exposure for 30 min at 10^4 cfu/mL and within the hemolymph after exposure for 2 h at 10^6 cfu/mL. Beetles internalized an average of 9.5×10^1 and 3.2×10^3 after a 2-h exposure to 2×10^4 and 2×10^6 cfu/mL, respectively. The lesser mealworm is a serious pest within the poultry brooder and laying industry and because of their mobility, voracious feeding habits, and prey potential may represent an active source facilitating the dissemination of *Salmonella*.

Key Words: Lesser mealworm—Darkling beetle—*Alphitobius diaperinus*—*Salmonella*—Poultry—Internalize.

Introduction

In poultry broiler production facilities, birds are in continuous contact with litter. A poultry house floor is neither sterile nor static, but rather it is a dynamic environment and a site for the potential transfer of various microbes between the birds and other organisms that inhabit the litter and soil. One of the most abundant insect species recovered from broiler chicken and turkey litter samples is the lesser mealworm, *Alphitobius diaperinus* (Panzer). All life stages of this insect can be found inhabiting and feeding within the litter of commercial poultry operations (Axtell and Arends 1990, Pfeiffer and Axtell 1980, Rueda and Axtell 1997, Stafford et al. 1988). These insects are omnivorous scavengers that feed on manure, spilled chicken feed, cracked eggs, chicken carcasses, house fly maggots, and detritus. In turn, they are often fodder for chickens, wild birds, and opportunistic rodents within the facility (Axtell and Arends 1990, Pfeiffer and Axtell 1980, Rueda and Axtell 1997), and cannibalism occurs among the beetles themselves in crowded conditions (Axtell 1994).

The epidemiology of *Salmonella* within a poultry facility is complex and not yet fully understood. In a 26-month study, *Salmonella enteritidis* persisted in the litter, feces, and feed after removal of birds from free-range paddocks and the beetle, *Amara aulica*, was found to still be contaminated 26 months after removal of birds from a closed layer (Davies and Breslin 2003). In a study by Harein et al. (1972) within a turkey brooder house, 27% of the lesser mealworm samples collected were positive for *Salmonella*. Lesser mealworms are a difficult pest to control and are often inadvertently dispersed to neighboring residences by the spreading of beetle containing manure to nearby fields (Armitage 1986, Calibeo-Hayes et al. 2005). These factors make them potential participants in the dissemination of pathogenic bacteria around the poultry house environment. Lesser mealworm beetles have previously been implicated in the transmission of several disease agents, including viral, fungal, and bacte-

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rial microbes (De las Casas et al. 1973, De las Casas et al. 1972, De las Casas et al. 1976, Despins et al. 1994, McAllister et al. 1995, McAllister et al. 1994, McAllister et al. 1996, Watson et al. 2000).

The harboring of bacteria internally, not just externally, by these pests further complicates the dilemma facing producers to limit the spread of infectious pathogens. Internalization of pathogenic bacteria might allow the establishment of a viable colony and long-term dispersal of bacteria into the environment by the beetle. Data exhibiting definitive evidence of internal carriage and threshold levels of bacterial contamination that contribute to dissemination is lacking. We used a validated external disinfection method allowing the differentiation of internally carried bacteria for this study (Crippen and Sheffield 2006). The use of *Salmonella* with a chromosomally inserted fluorescent tag allowed the long-term tracking of specifically introduced bacteria within the gastrointestinal tract (GIT) of the beetle. The concentration and time thresholds for the oral acquisition of *Salmonella* by lesser mealworm beetles were assessed and demonstrated that given favorable conditions, beetles were capable of rapidly internalizing *Salmonella* from relatively low levels of environmental contamination.

Materials and Methods

Beetles

The Southern Plains Agricultural Research Center (SPARC) starter colony of *A. diaperinus* was a generous gift from a colony originally isolated from a poultry farm located in Wake County, NC and maintained by Dr. D. W. Watson (North Carolina State University, Raleigh, NC). The SPARC colony was initiated and has remained in production since 2004. Beetles were reared in 1000 mL wheat bran (Morrison Milling Co., Denton, TX) in plastic containers ($15 \times 15 \times 30$ cm) with screen tops and held at 30°C in an 8:16-h (light:dark) cycle. Additionally, each cage contained a 6-cm^2 sponge moistened with deionized water and a 0.5-cm-thick slice of a medium-sized apple replenished twice per week, and 30 mL of fishmeal (Omega Protein, Inc., Hammond, LA) was added to the wheat bran once per week.

Experimental design

Three replications of each experimental exposure protocol were conducted using 10 beetles per time point and concentration. The beetles of at least 4 weeks of age were selected from the colony and exposed to a lawn of bacteria in exposure tubes. These exposure tubes were produced by placing 7 mL trypticase soy agar into a 17×100 mm (14 mL) snap cap polypropylene round bottom tube (Fisher Scientific, Pittsburgh, PA). A fluorescent marker bacteria, *Salmonella enterica* serovar Typhimurium-green fluorescent protein (ST-GFP; a generous gift from Drs. Roy J. Bongaerts and Jay Hinton, Norwich Research Park, Norwich, UK) was used to track the location of bacteria within the beetle (Hautefort et al. 2003).

Exposure threshold determinations. Phosphate-buffered saline (10 μL; control) or ST-GFP (treated) was added to the top of the agar at appropriate concentrations for study A: $1.12 \times 10^2 \pm 1.5 \times 10^1$ (10^2), $1.05 \times 10^3 \pm 3.9 \times 10^2$ (10^3), $7.53 \times 10^3 \pm 8.4 \times 10^2$ (10^4) colony-forming units (cfu)/mL, and study B: $1.21 \times 10^3 \pm 1.5 \times 10^2$ (10^3), $1.06 \times 10^5 \pm 1.3 \times 10^4$ (10^5), and $5.78 \times 10^6 \pm 4.9 \times 10^5$ (10^7) cfu/mL. Two beetles were added per tube and allowed to move freely at 30°C in the dark. Five tubes in total were used for each time point and concentration combination. Beetles were then harvested at appropriate time points for study A (0.5 and 2 h) and study B (2, 6, and 12 h). Salmonella Typhimurium (ST) has a rapid growth curve. In order to ensure a consistent exposure level for beetles throughout the time course, beetles were transferred into a new exposure tubes made each hour for the duration of the exposure time. Postexposure, ST-GFP was cultured from a random sample of exposure tubes to ensure that the beetles were exposed to viable bacteria. Beetles were disinfected by sequential wash in 95% ethyl alcohol (EtOH), and SporGon® (Decon Labs, Inc. Bryn Mawr, PA), and disinfection was validated by exposure to and culture of the washed beetle in buffered peptone water (BPW) as previously described (Crippen and Sheffield 2006).

Dissection. Following exposure to marker bacteria and surface disinfection, the study insects were dissected. Beetles were pinned dorsally through the thorax, immediately to the right of the midline, and onto a bed of dissecting wax using a sterile stainless steel insect pin. The elytra and wings were carefully removed, a small hole was punctured into the dorsal abdominal cuticle, and hemolymph was collected into sterile capillary tubes. The hemolymph was expelled into 1 mL BPW for enrichment. Following collection of hemolymph, the abdominal and thoracic cuticles were removed to expose the entire alimentary canal. The external genital sclerites were detached and pulled upward to lift the alimentary canal from the body cavity. The head was then carefully removed from its attachments to the thorax, ensuring that it did not tear from the esophagus, and the entire alimentary canal was resected from the beetle. The alimentary canal was placed onto a separate sterile area and the head was removed from the esophagus and placed into 1 mL BPW and 300 mg of low binding silica beads (BT & C/OPS Diagnostics, Bridgewater, NJ) to assist in disruption by vortexing. The remaining alimentary canal was placed into 1 mL BPW. Each vial was vortexed vigorously for 15–30 s then incubated at 39°C for 18–24 h. A 0.1-mL aliquot of this enrichment was spread onto brilliant green agar (BGA) plates with 1.2% chloramphenicol at 37°C for 18–24 h. After isolation by selective culturing on BGA plates, the presence of ST-GFP was confirmed using a Leica DMLB fluorescent microscope equipped with a 100× oil lens and a fluorescein isothiocyanate (FITC) filter pack.

Enumeration of Uptake. In study C, 10 μL of PBS (control) or ST-GFP (treated) was added to the top of the agar at appropriate concentrations (2.03×10^2, 2.20×10^4, and 2.09×10^6 cfu/mL). Beetles were exposed as described above and harvested after 2-h exposure. Postexposure, beetles were disinfected by sequential wash in 95% EtOH and SporGon® (Decon Labs, Inc., Bryn Mawr, PA), and disinfection was validated by exposure to and culture of the washed beetle in BPW as previously described. Following exposure to marker bacteria and surface disinfection, the study insects were homogenized in brain heart infusion broth (Neogen Corp., Lansing, MI). Enumeration was performed by serial dilution serial onto BGA plates with 1.2% chloramphenicol and incubation at 37°C for 18–24 h.

Data analysis. Data were analyzed using commercially available statistical software (SAS, SAS Institute Inc., Cary,

Table 1. Study A: Results of ST-GFP Isolation from Beetles Feeding on Various Concentrations of Bacteria on Agar for 30 min to 1 h (Mean Percentage of Beetles Positive for ST-GFP)

	Hemolymph		*Head*		*GIT*	
Dose	*30 min*	*1 h*	*30 min*	*1 h*	*30 min*	*1 h*
PBS*	0 ± 0‡	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
10^{2}†	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
10^{3}	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
10^{4}	0 ± 0	0 ± 0	3 ± 6	0 ± 0	10 ± 6	30 ± 10

*Beetles exposed to 10 μL of PBS.
**Beetles exposed to 10 μL of ST-GFP at 10× colony-forming units/mL.
‡Mean of three replications of $n = 10$ beetles ± standard deviation.
ST-GFP, *Salmonella enterica* serovar Typhimurium-green fluorescent protein; GIT, gastrointestinal tract; PBS, phosphate-buffered saline.

NC). Descriptive statistics were generated and presented in table formats. Within each time point and sample type, a means comparison of exposure concentration was performed using the GLIMMIX Procedure to determine least square means ($p < 0.01$). The geometric mean (GM) was calculated as the nth root of the product of n numbers (Microsoft Office Excel 2003, Microsoft Corp.).

Imaging of internalization of bacteria

Beetles were randomly selected from the colony for exposure to bacteria for imaging purposes. The beetles were exposed to a lawn of bacteria in exposure tubes as described above. Briefly, 10 μL of 10^{6} cfu/mL ST or 10^{6} cfu/mL ST-GFP was added to the top of the agar and incubated prior to placement of beetles onto the agar. Beetles were then placed in the tubes and exposed at 30°C for 16–18 h. The beetle alimentary canal was excised from the beetle *in toto* as described above. The tract was frozen for preservation by covering with Tissue-Tek® O.C.T. Compound (Ted Pella, Inc, Redding, CA) on an aluminum planchet. The planchet was transferred to a liquid nitrogen cooled metal block where the O.C.T. compound was allowed to freeze. The frozen tissue was removed from the planchet, transferred to a cryovial, and sealed in a plastic bag for storage at −80°C. Prior to observation, an individual GIT was thawed in saline.

For epifluorescent imaging, once thawed the GIT was rinsed three times in fresh saline then stretched onto a microscope slide in a drop of saline. The fluorescent images were collected using an FITC filter on a Olympus SZX12 fluorescent dissecting scope through a 7× lens and a Olympus DP70 camera.

For sequential fluorescent imaging, once thawed the GIT was rinsed three times in fresh saline and cut into approximately 3-mm segments. Each segment was subsequently sliced by hand using a double-edge razor blade in a drop of saline on a microscope slide. The tissue slices were coverslipped and viewed using a Zeiss Stallion Double Detector Imaging system with Photometrics Coolsnap HQ CCD cameras (Carl Zeiss Microimaging, Thornwood, NY). Bacteria were sequentially imaged using a GFP/Alexa 488 filter set followed by differential interference contrast optics using a C-Apochromat (63× water correction; 1.2 NA) objective lens.

Results

Study A was performed to determine a lower threshold concentration level and exposure duration for the internal uptake of *Salmonella* by the beetles. The beetles were exposed to 10 μL of PBS, or 10^{2}, 10^{3}, or 10^{4} cfu/mL ST-GFP for 0.5 or 1.0 h, then surface disinfected, dissected, and cultured (Table 1). Uptake was detected at the 10^{4} cfu/mL exposure concentration only and occurred in the GIT of 10 and 30% of beetles exposed for 0.5 and 1 h, respectively. A few beetles, 3%, retained ST-GFP in the head section after a 0.5-h exposure. No marker bacteria were found in the hemolymph at these lower exposure times and concentrations.

Study B was performed to determine concentration levels and exposure durations at which all beetles would consistently internalize *Salmonella*. The beetles were exposed to 10 μL of PBS, 10^{3}, 10^{5}, or 10^{7} cfu/mL ST-GFP for 2, 6, or 12 h, then surface disinfected, dissected, and cultured (Table 2). There were no major differences found between time of exposure within each exposure concentration level; however, there were significant difference in relation to concentration of ST-GFP exposure within each time point ($p < 0.01$) and within sample type (hemolymph, head, and GIT).

Exposures to 10^{7} and 10^{5} cfu/mL resulted in the ST-GFP infiltration of the GIT in all beetles exposed for 2 h or more. Nearly

Table 2. Study B: Results of ST-GFP Isolation from Beetles Feeding on Various Concentrations of Bacteria on Agar for 2 to 12 h (Mean Percentage of Beetles Positive for ST-GFP)

	Hemolymph			*Head*			*GIT*		
Dose	*2 h*	*6 h*	*12 h*	*2 h*	*6 h*	*12 h*	*2 h*	*6 h*	*12 h*
PBS*	0 ± 0‡,a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a
10^{3}†	0 ± 0a	0 ± 0a	3 ± 6a,b	30 ± 26a,b	37 ± 6b,c	50 ± 26c,d	80 ± 10b	87 ± 15c	93 ± 6c
10^{b}	13 ± 6a,b,c	7 ± 6a,b	27 ± 12c	83 ± 0.6d,e	70 ± 1.7d	83 ± 1.5e,f	100 ± 0c	100 ± 0c	100 ± 0c
10^{7}	23 ± 21a,b,c	30 ± 17c	30 ± 26c	100 ± 0†	83 ± 15e,†	100 ± 0†	100 ± 0c	100 ± 0c	100 ± 0c

*Beetles exposed to 10 μL of PBS.
†Beetles exposed to 10 μL of ST-GFP at 10× colony-forming units/mL.
‡Mean of three replications of $n = 10$ beetles ± standard deviation.
a,b,c,d,e,fWithin each sample type (hemolymph, head, and GIT), means with the same letter are not significantly different.
ST-GFP, *Salmonella enterica* serovar Typhimurium-green fluorescent protein; GIT, gastrointestinal tract; PBS, phosphate-buffered saline.

all of these beetles also retained ST-GFP in the head section, which included the mouth parts. At 10^3 cfu/mL exposure, only 80% of the beetles harbored ST-GFP in the gut at 2 h, which increased to 93% by 12 h. The ST-GFP was also detected in the hemolymph of a small percentage of the insects; the number of beetles contaminated increased with exposure concentration.

Study C was performed to enumerate the uptake of ST-GFP by the beetles. The beetles were exposed to an average of 10 μL of PBS, 2×10^2, 2×10^4, or 2×10^6 cfu/mL ST-GFP, surface disinfected, homogenized, cultured by serial dilution, and enumerated to quantify the amount of marker bacteria internalized by the beetle over a 2-h period (Table 3). Exposure to 2×10^4 cfu/mL resulted in internal uptake by 70% of the beetles at a mean level of 9.45×10^1 cfu/beetle and exposure to 2×10^6 cfu/mL resulted in internal uptake by 100% of the beetles a mean level of 3.15×10^3 cfu/beetle.

Beetles were exposed to 2×10^6 cfu/mL ST-GFP for 16–18 h and the alimentary canal was excised for microscopic imaging. Fluorescent imaging by dissection microscopy demonstrated green fluorescence throughout the alimentary canal of the beetle following exposure to ST-GFP. Control beetles, exposed to ST only, demonstrated background fluorescence. Representative specimens are shown in Fig. 1.

Excised alimentary canals of ST-GFP-exposed beetles were also sequentially imaged by fluorescent and differential interference contrast optics to confirm that the fluorescent emission radiating from the alimentary canal emanated from bacteria. GFP fluorescent bacteria were present in the fore-, mid-, and hindgut sections (data not shown) of the alimentary canal. The hindgut is defined as the tract from the genital sclerites anterior to the proctodeal valve. The midgut is the tract from the proctodeal valve anterior to the stomodeal valve. The foregut consisted of the tract from the stomodeal valve anterior to the esophageal attachment at the back of the cranium. Figure 2A is a representative specimen of a foregut section establishing that bacteria are present on the internal gut wall of the beetle's alimentary canal. Figure 2B captured the green fluorescent emission upon 488-nm excitation of the same section demonstrating that the green fluorescence was specific to the bacteria present.

Discussion

Litter beetles, especially the lesser mealworm, have become serious pests within the poultry brooder and laying industry. It is suspected that this pest harbors and disseminates a number of pathogens that could affect bird health and performance. These beetles have high reproductive rates, are difficult to control, and because of their mobility, feeding habits, and prey potential, are implicated as mechanical vectors for diseases, such as Mareks disease, avian influenza, bacterial diseases, fowl pox, coccidiosis, and New Castle disease (Brown et al. 1992, Hald et al. 1998, Harein et al. 1972, Harein et al. 1970). Ultimately, the lesser mealworm is portrayed as a reservoir source contributing to the persistence and transmission of pathogens to individual birds within a poultry production facility.

Comprehensive knowledge of the pathways involved in the infection of poultry with bacterial pathogens remains unclear, including the role of lesser mealworm beetles in long-term pathogen retention and transmission. Previous studies have investigated their role as carriers of microbes (De las Casas et al. 1972, De las Casas et al. 1968, Gray et al. 1999, Hald et al. 1998, Harein and De las Casas 1968, Harein et al. 1970, McAllister et al. 1995, McAllister et al. 1994, McAllister et al. 1996). In order to institute viable biosecurity control measures, an in-depth understanding of the relationship between these pathogens, their insect vectors, and the individual birds is required. The aim of this study was to quantify (1) the internal acquisition of *Salmonella* by lesser mealworms, (2) the minimal exposure duration and concentration necessary for oral uptake and internal retention, and (3) the exposure duration and concentration required for 100% infection of the beetles.

Previous studies have evaluated the carriage of bacteria by the lesser mealworm without regard to separating internal from external sources (Bates et al. 2004, Goodwin and Waltman 1996, Skov et al. 2004, Strother et al. 2005, Templeton et al. 2006). Other studies have used various methods to externally disinfect beetles prior to assessing contamination of the beetles (De las Casas et al. 1968, Harein et al. 1972, Harein et al. 1970, McAllister et al. 1996). However, previous work by this lab demonstrated that many of the external disinfection methods used were inadequate to completely rid the external surface of the insect of bacteria (Crippen and Sheffield 2006). Therefore, conclusions drawn about the source of pathogenic contamination originating from internally carried bacteria remained unclear.

De las Casa et al. (1968) used a sodium hypochloride-based procedure to surface disinfect larvae and then exposed the

Table 3. Mean Internal Concentration of ST-GFP per Beetle after 2h Exposure

Dose (cfu/mL)	*Beetles (positive/n)*[†]	*GM*[‡] *(cfu/beetle)*	*Upper 95% CI*[§] *(cfu/beetle)*	*Lower 95% CI (cfu/beetle)*
PBS	0/3	0	0	0
2.03×10^2	0/3	0	0	0
2.20×10^4	21/30	9.45×10^1	15.40×10^1	1.39×10^1
2.09×10^6	30/30	3.15×10^3	4.68×10^3	1.44×10^3

*Agar dose with 10 μL ST-GFP at this concentration level.
[†]Number of beetles positive for ST-GFP/total number of beetles exposed.
[‡]Geometric mean ST-GFP acquired by beetles.
[§]95% CI of the median.
ST-GFP, *Salmonella enterica* serovar Typhimurium-green fluorescent protein; PBS, phosphate-buffered saline; CI, confidence interval; cfu, colony-forming units; GM, geometric mean.

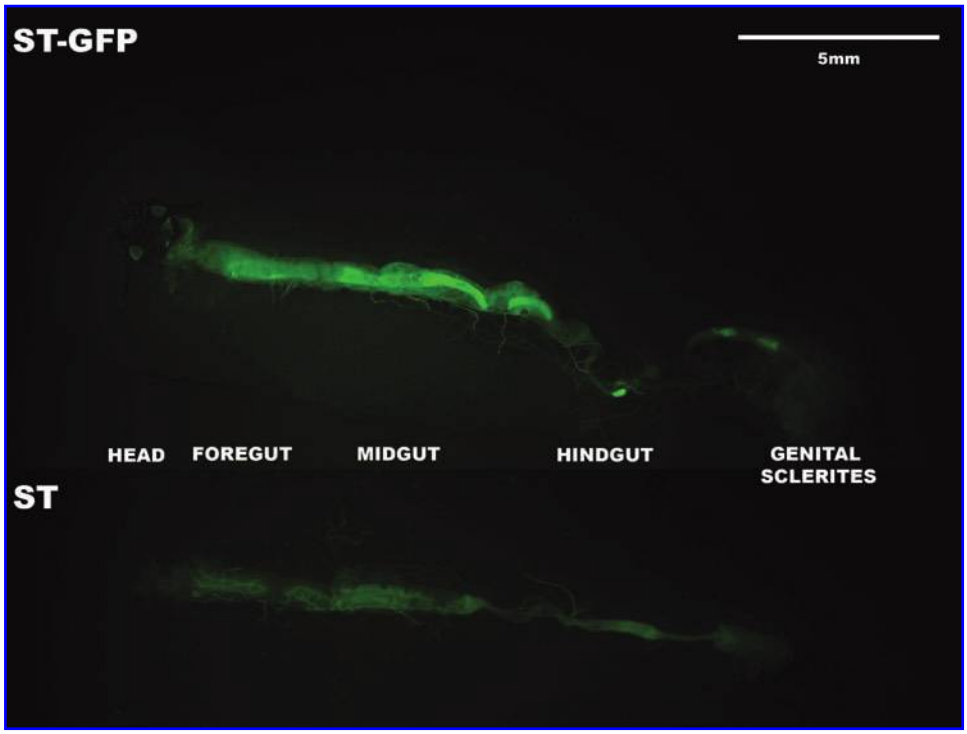

FIG. 1. The beetles were exposed to 2×10^6 colony-forming units/mL *Salmonella enterica* serovar Typhimurium-green fluorescent protein (ST-GFP) or serovar Typhimurium (ST) for 16–18 h and the alimentary canal was excised for analysis. The fluorescent images were collected using a fluorescein isothiocyanate filter on a fluorescent dissecting scope.

insects to bacterial growth media to identify and subsequently remove any contaminated insects from the study. They determined that about 50% of the insects subjected to this surface disinfection method were still contaminated with microbes. The insects were then surface disinfected a second time following exposure to ST and prior to maceration to determine internal carriage of *Salmonella*. However, checks on the effectiveness of the second surface disinfection were done only "periodically" and only on "some insects." Therefore, some of the subsequent bacterial counts reported could have originated from residual external contamination of the insects. In our study, the effectiveness of the surface disinfection was assessed on every insect prior to determination of presence of internalized *Salmonella*. Harein et al. (1972, 1970) used a similar hypochlorite surface disinfection technique with subsequent exposure to bacterial growth media as a disinfection check and isolated *Salmonella* from 2.2% and 27% of the beetle samples collected at two different poultry brooder facilities. McAllister et al. (1996) used a 70% ethanol surface disinfection technique prior to maceration of the beetle; however, the effectiveness of the surface disinfection was not determined after washing. They isolated *Salmonella* from >70% of beetles fed in the laboratory for 24 h on chicken feed contaminated with 3×10^8 cfu/mL.

Davies and Wray (1995) reported on artificial exposure of the lesser mealworm beetle to fish meal contaminated with

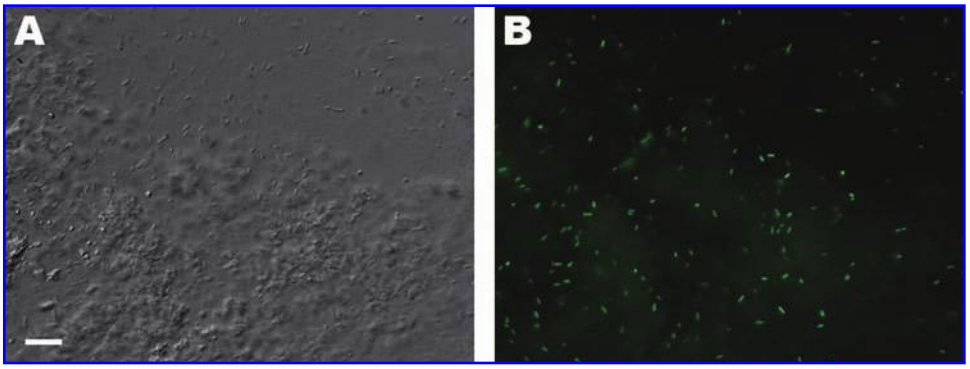

FIG. 2. The beetles were exposed to 2×10^6 colony-forming units/mL *Salmonella enterica* serovar Typhimurium-green fluorescent protein for 16–18 h and the alimentary canal was excised for analysis. The tissue was then sectioned and sequentially imaged. (**A**) Contrast optics image of a foregut section. (**B**) Fluorescent emission captures using an fluorescein isothiocyanate filter from the identical foregut section. Bar = 10 μm.

10^3 to 10^4 cfu *Salmonella* g^{-1}. They hypothesized that *Alphitobius* beetles may be relatively resistant to *Salmonella* uptake after they found negative results throughout a 6-day study. Unfortunately, no data were given demonstrating the viability of the bacteria within the fish meal itself over the 6-day period and no description of the exposure container size was provided. In stark contrast, our study demonstrates that these beetles are very capable of acquiring *Salmonella* internally. The beetles were exposed on agar while confined to a 17-mm diameter space to ensure contact. In addition, viability of bacteria within the exposure tubes was validated postexposure. Under these conditions, the beetles did acquire bacteria internally. Using a previously validated external disinfection technique for this study (Crippen and Sheffield 2006), we determined that lesser mealworm beetles could acquire *Salmonella* in their GIT within 30 min of exposure to 10^4 cfu/mL, the shortest time point evaluated. After a 2-h exposure to 10^3 cfu/mL *Salmonella*, an average of 80% of the beetles had *Salmonella* in their GIT. This contamination level increased after 12 h of exposure. Beetles feed frequently, and presumably the increased contamination rate is a result of beetles feeding during the intervening 10 h.

In addition, every beetle exposure to 10^5 cfu/mL acquired *Salmonella* internally within 2 h. Fewer beetles had ST-GFP in the head section, although the concentration curve followed a similar pattern as that found in the GIT. The reduction of *Salmonella* found in the head section could be due to the external disinfection procedure. The beetles die during this procedure; therefore, if the buccal cavity was open, then the disinfectants would have access to bacteria in that region. In addition, food stuffs are likely to pass fairly rapidly out of the buccal cavity and move deeper into the alimentary canal. Furthermore, it is not known whether these beetles produce saliva with antimicrobial properties within the buccal cavity. Isolation of *Salmonella* from the hemolymph at these exposure levels could indicate extraintestinal dissemination and warrants further investigation. From a management standpoint, it is important to determine if this insect is simply a mechanical vector or a reservoir that can sustain and support a colony of *Salmonella* internally.

Although conditions in the poultry house environment would not restrict the insect's movements, these beetles are known to congregate in great numbers under and around feed and water stations (Axtell and Arends 1990). It is difficult to mimic, in controlled laboratory studies, the many environmental and confounding factors that influence contamination and infection in the field. However, given that in this study, uptake occurred after exposure to as little as 10 μL of 10^4 cfu/mL for 30 min, it is likely that such conditions could occur in poultry house litter and fecal material deposited at the feed- and water-dispensing sites where beetles spend a great deal of time. Harborage of *Salmonella* internally by larvae and beetles that migrate into wall spaces is an added biosecurity threat. *Salmonella* may not be cleared from the house by litter cleanout if insects still within the walls of the house maintain the infestation between flock rotations. Studies to determine the levels of bacteria and the duration of infestation within the GIT are ongoing. However, it is difficult to separate true colonization of the GIT from repeated external contamination by exposure to bacteria excreted by the beetle.

Understanding the dynamics of movement of pathogenic bacteria within the environment and between species is crucial to unraveling the epidemiology of diseases and to developing control measures to reduce or eliminate the inadvertent propagation and dissemination of pathogens between flocks or to nearby fauna. Dissemination by surface contamination demonstrates that these beetles are capable of acting as interim transport hosts. However, the harboring of bacteria internally, not just externally, by these pests opens the question of whether *Salmonella* will colonize within these insects and act as a reservoir that could produce pathogens over time. We have demonstrated that relatively short exposures to low concentrations of *Salmonella* result in the acquisition of viable bacteria internally by this beetle. Current farm management practices can perpetuate persistent facility infestations and contribute to the dispersal of beetles and any pathogens they may harbor.

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